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Oxidation of low density lipoproteins from patients with renal failure or renal transplants

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Oxidation of low density lipoproteins from patients with renal failure or a renal transplant. Peroxidation of low density lipoproteins (LDL) may be involved in the development of atherosclerosis which is prevalent in patients with chronic renal failure and renal transplant recipients. We determined the copper ion catalyzed oxidation *in vitro*, vitamin E content, and chemical and fatty acid composition of LDL isolated from 38 patients with renal disease and 15 healthy subjects. Also the acute effect of hemodialysis treatment on LDL oxidation variables was tested. The lag time in conjugated diene formation during oxidation was significantly ($P = 0.011$) shorter in LDL from renal transplant recipients (66 min, $N = 18$) mainly due to significantly ($P < 0.05$) shorter times in women (47 min, $N = 7$), compared with healthy subjects (83 min, $N = 15$), patients on hemodialysis (91 min, $N = 13$) and patients treated by continuous ambulatory peritoneal dialysis (CAPD) (82 min, $N = 7$). The maximum rate and the extent of LDL oxidation were significantly ($P < 0.01$) lower in all patients with renal disease compared with healthy subjects. The triglyceride content of LDL was significantly ($P < 0.001$) higher in women with kidney grafts (7.3%) compared with levels in the corresponding men (5.3%) and healthy women (5.0%), and was correlated significantly with the lag time in LDL oxidation in renal transplant recipients (Spearmans $r = -0.502$, $P = 0.034$). The percentage oleic acid in LDL was significantly higher ($P = 0.002$) and the percentage linoleic acid was significantly lower ($P = 0.046$) in patients with renal disease, and may largely account for their lower rates and extent of LDL oxidation. Levels of the LDL oxidation variables and organic lipid peroxide content of LDL were not significantly different before and after hemodialysis and 24 hours later. These results suggest that LDL from women with renal transplants may be abnormally susceptible to oxidation possibly due to increased triglyceride content.

Patients with chronic renal failure have a substantially increased risk of death from cardiovascular disease compared with age-matched individuals from the general population [1]. Furthermore, the risk of coronary heart disease (CHD) remains high in patients treated by hemodialysis [2] and kidney grafting [3]. Thus treatments to counteract uremia do not decrease and may even increase CHD risk in patients with renal insufficiency. Factors responsible for the development of atherosclerosis in chronic renal failure are not well known. Potentially atherogenic patterns of lipid abnormalities are seen in patients receiving maintenance hemodialysis including hypertriglyceridemia, increased levels of very low density lipoproteins (VLDL) and intermediate density lipoproteins (IDL), low levels of high density lipoproteins (HDL) and enrichment of low density lipoproteins (LDL) with triglycerides [4, 5]. In renal transplant recipients raised levels of plasma triglycerides, cholesterol, VLDL and LDL have been noted [6]. Hypertension and glucose intolerance are also associated with chronic renal failure and may contribute to the lipid abnormalities and to the increased risk of CHD in affected individuals.

Recently, lipid peroxidation [7] and particularly the oxidative modification of LDL [8] have been postulated as important factors in the development of atherosclerosis. Increased levels of lipid peroxides [7] and autoantibodies to oxidatively modified LDL [9] are detected in patients with atherosclerotic disease. Also, the susceptibility of LDL to oxidation *in vitro* has been associated with the severity of coronary atherosclerosis in patients with CHD undergoing angioplasty [10]. Studies indicating that antioxidants are capable of retarding the development of atherosclerosis in animals [11, 12] further support a role for lipid oxidation in the formation of arterial lesions. Oxidized LDL has several properties which are potentially atherogenic. For example, oxidatively modified LDL promotes accumulation of cholesteryl esters in cultured macrophages [8] reminiscent of foam cell formation which is a key step in the development of the atherosclerotic lesion. In addition oxidized LDL are cytotoxic [13] and are an attractant for macrophages [14].

There is evidence that oxidative stress is increased in patients with chronic renal failure. Raised levels of lipid peroxidation products are found in plasma from uremic patients [15, 16] and during hemodialysis [17], and activation of oxidative metabolism in polymorphonuclear leukocytes by the dialysis membrane has been observed [18]. These conditions could increase the susceptibility of LDL to oxidation. Recent studies have reported increased susceptibility to oxidation of LDL isolated from patients with chronic renal failure treated by hemodialysis or continuous ambulatory peritoneal dialysis (CAPD) [19]. Reduced efficiency of the antioxidant vitamin E in LDL was also detected and may contribute to this increased susceptibility of hemodialyzed LDL to oxidation [20]. The acute effect of hemodialysis on the oxidation of isolated LDL was not tested. In renal transplant recipients, treatment with cyclosporine can increase the susceptibility of isolated LDL to oxidation if blood levels of the drug are high enough [20]. However, other factors including LDL lipid and fatty acid composition could influence oxidation of LDL from patients with chronic renal failure or kidney transplants. Circulating LDL

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is triglyceride-enriched in patients with renal failure [4], and high LDL triglyceride content is associated with increased susceptibility of LDL to oxidation *in vitro* [10]. Fatty acid composition also influences the oxidation of isolated LDL with increased content of polyunsaturated fatty acids enhancing the susceptibility of LDL to oxidation and increased content of monounsaturates having the opposite effect [21, 22]. Furthermore, altered dietary intake of these fatty acids leads to predictable changes in the oxidation of isolated LDL [22–24]. Renal failure is frequently associated with changes in diet which could conceivably alter fatty acid composition and oxidation of LDL from these patients.

The aim of the present study was to compare the susceptibility to oxidation and the lipid, fatty acid, lipoperoxide and vitamin E content of LDL among renal transplant recipients, patients with chronic renal failure treated by hemodialysis or CAPD and healthy subjects. In addition, oxidation of LDL was measured immediately before, at the end, and 24 hours after hemodialysis to test the acute effect of this procedure on LDL oxidation. In this study we have used the terms "susceptibility or resistance to oxidation" to refer to the delay in onset of LDL oxidation (lag time) and "oxidizability" for the maximum rate of oxidation.

Methods

Subjects

Thirty-eight patients with chronic renal failure or kidney transplants (ages 19 to 78 years) were recruited from patients attending the Otago Nephrology Unit. Patients with nephrotic syndrome and patients receiving lipid-lowering therapy were excluded. Thirteen patients were on hemodialysis, seven were treated by CAPD and 18 were renal transplant recipients. The etiology of renal failure in the patients was as follows: glomerulonephritis ($N = 22$), reflux nephropathy ($N = 5$), hypertension ($N = 4$), autosomal dominant polycystic kidney disease ($N = 2$), diabetes mellitus ($N = 2$) and other causes ($N = 3$). Eight patients had evidence of ischemic heart disease (IHD) which was diagnosed on the basis of clinical symptoms and signs, along with ECG or echocardiographic changes or both. None of these patients had suffered an ischemic event. Eighteen patients were receiving cardiovascular medications. Angiotensin converting enzyme (ACE) inhibitors and/or dihydropyridine calcium channel antagonists were most frequently used to treat hypertension. Two patients were taking a beta-blocking drug. Most of the dialysis patients were taking calcium carbonate as a phosphate binder and 1-α-calcitriol supplementation. Two patients were receiving erythropoietin subcutaneously. Renal transplant recipients received azathioprine and prednisone ("double therapy," $N = 5$) or both these drugs and cyclosporine A ("triple therapy," $N = 11$) or cyclosporine A and prednisone ($N = 2$) to prevent rejection of the kidney graft. Patients treated by dialysis were either on home hemodialysis or CAPD. Hemodialysis patients were dialyzing three times weekly (12 to 18 hr/week) using cellulose acetate hollow fiber dialyzers and acetate dialysate. The usual length of a hemodialysis procedure was five hours. The Baxter Disconnect system was used by CAPD patients. Dietary protein intake was not restricted in any patient group and none of the patients was taking antioxidant supplements. Hemodialysis patients were advised to restrict intake of potassium-rich foods and fluid where necessary, but were not given any other dietary advice. Six patients including including three hemodialysis, one CAPD and two renal transplant recipients smoked; they were all men.

Fifteen healthy subjects (ages 31 to 52 years) were recruited from staff of the Dunedin Public Hospital and University of Otago. None was taking medication or antioxidant supplements and none smoked.

The study was approved by the Ethics Committee of the Otago Area Health Board and participants gave written and informed consent.

Lipoprotein separation

Venous blood was taken after an overnight fast and was collected in vacutainer tubes containing disodium EDTA (1.5 mg/ml). Blood was collected from hemodialysis patients immediately before a dialysis session, at the end of dialysis and at the 24 hour point. Plasma was separated by low-speed centrifugation at 4°C. Sequential ultracentrifugation (20 and 24 hr at 40,000 rpm in a Beckman 50.3 Ti rotor at 4°C) of plasma at densities 1.019 g/ml and 1.063 g/ml was used to separate LDL (d 1.019 to 1.063 g/ml) and VLDL+IDL (d 1.019 g/ml) fractions. LDL was dialyzed against phosphate buffered saline (PBS) pH 7.4 and then PS containing EDTA (5 μmol/liter) overnight at 4°C. The lipoprotein was stored in the dark at 4°C and was used within three days. Protein in LDL was measured by the Lowry method [25]. High density lipoprotein cholesterol was measured in the supernatant after precipitation of apoB-containing lipoproteins with dextran sulphate/magnesium chloride [26]. Cholesterol and triglycerides were measured in plasma and lipoprotein fractions using enzymatic kits and calibrator supplied by Boehringer Mannheim Ltd (Germany). In addition free cholesterol and phospholipids were measured in LDL using enzymatic kits (Boehringer Mannheim Ltd). Plasma lipoprotein (a) was measured using a two-site radioimmunoassay kit (Pharmacia). Whole blood and plasma cyclosporine levels were measured in the laboratories of Dunedin Public Hospital by fluorescent polarization immunoassay using a commercial kit (Abbott TDX).

Oxidation of LDL

Copper ions were used to oxidize the isolated LDL, and the formation of conjugated dienes was measured to monitor oxidation. Conjugated diene formation was determined essentially as described by Sattler and coworkers [27]. To a quartz cuvette was added PBS (2 ml) which had been aerated for 15 minutes, an aliquot of LDL solution containing 50 μg LDL protein and 5 μmol/liter EDTA in PBS to bring the total volume to 2.2 ml. After the addition of 32 μl of 1 mmol/liter copper sulfate the absorbance at 234 nm was monitored at 10 minute intervals. Quantities of conjugated dienes formed were calculated using the molar extinction coefficient (29500 liter/mol/cm) for conjugated dienes. The lag time was obtained from the intersection of lines drawn through absorbance values before oxidation started (lag phase) and during the following rapid oxidation (propagation phase) of LDL. The rate of conjugated diene formation during the propagation phase and also the maximum level of dienes attained were calculated.

Fatty acid composition

Heptadecanoic acid (20 μl of a 1.15 mg/ml solution in toluene) internal standard was added to an aliquot (0.2 ml) of LDL solution (protein 0.3 to 0.9 g/liter). The mixture was extracted

Table 1. Clinical characteristics and plasma lipids and lipoprotein concentrations in the subjects

	HD $N = 13$	CAPD $N = 7$	RT $N = 18$	Healthy subjects $N = 15$	ANOVA P
Clinical features					
Age *years*	51 ± 19	65 ± 7[abd]	42 ± 13[c]	43 ± 7	0.001
Gender (*M/F*)	11/2	6/1	11/7	5/10	
Body weight *kg*	67.2 ± 12.3	71.1 ± 5.2	71.3 ± 14.5	69.4 ± 12.0	NS
BMI kg/m^2	22.2 ± 2.9	25.5 ± 1.7	24.9 ± 5.0	25.0 ± 4.0	NS
Duration of treatment *months*	30 ± 33	36 ± 39	60 ± 42		NS
Plasma creatinine *μmol/liter*	741 ± 301	751 ± 129	158 ± 68[cd]	ND	< 0.001
Plasma lipids and lipoproteins *mmol/liter*					
Cholesterol	5.50 ± 1.24	6.70 ± 1.57[ab]	6.41 ± 1.15[ac]	5.36 ± 0.57	0.01
VLDL + IDL cholesterol	1.31 ± 0.60[a]	1.77 ± 1.20[a]	0.86 ± 0.34[cd]	0.73 ± 0.46	0.001
LDL cholesterol	2.94 ± 0.89	3.38 ± 1.07	3.49 ± 0.87	2.78 ± 0.54	NS
HDL cholesterol	1.02 ± 0.54[ac]	1.01 ± 0.18[d]	1.62 ± 0.48	1.39 ± 0.36	0.001
Triglycerides	2.05 ± 0.87	2.12 ± 1.02	1.63 ± 0.59	1.42 ± 0.81	NS
VLDL + IDL triglycerides	1.60 ± 0.75	1.53 ± 0.95	1.20 ± 0.56	1.05 ± 0.77	NS
Lp(a) *U/liter*	489 ± 455	569 ± 479	237 ± 309	266 ± 273	0.07[e]

Values are mean ± SD. Abbreviations are: HD, hemodialysis; CAPD, continuous ambulatory peritoneal dialysis; RT, renal transplants; ANOVA, analysis of variance; Lp(a) lipoprotein (a); ND, not determined.

Significance $P < 0.05$ (Duncan's test: [a] vs. healthy subjects; [b] HD vs. CAPD; [c] RT vs. HD; [d] CAPD vs. RT

[e] Significance calculated on log transformed data

twice with chloroform/methanol (2:1) according to the Folch procedure [28]. The combined extracts were evaporated to dryness under nitrogen, then redissolved in a small volume of chloroform/methanol (2:1) and transferred quantitatively to a Teflon screw-cap tube. The solvent was again evaporated under nitrogen and toluene (1 ml) and 14% boron trifluoride/methanol (2 ml) were added. The tube was securely capped then heated on a boiling water bath for 90 minutes. At the end of this transesterification step, the tube was cooled, water (1 ml) was added and the fatty acid methyl esters were extracted twice with hexane. The hexane extracts were brought to dryness under a stream of nitrogen, redissolved in a small volume of dichloromethane and transferred to a small conical minivial. The solvent was evaporated under nitrogen and the residue was redissolved in 50 μl of dichloromethane. A 3 μl sample of this solution was injected onto a Chromopack CP Sil88 capillary column (50 m X 0.22 mm) in a Varian 3700 gas chromatograph with a split-ratio set at 1:10. Helium (0.8 kg/cm^2 column head pressure and flow rate 1.5 ml/min) was used as the carrier gas and the injector temperature was 260°C and the FID detector temperature was 280°C. Column temperature was programmed from 165°C to 210°C at 3°/min. Peak area was measured using a Spectra-Physics SP 4290 integrator. The peak quantification was based on peak area comparison with the internal standard and fatty acid composition was expressed in wt %. The concentrations of fatty acids in LDL (nmol/mg protein) were also calculated but are not reported.

Lipid peroxides

Organic lipid peroxide levels were measured in LDL prior to oxidation using the method of el-Sadaani and coworkers [29] modified to minimise binding of iodine to LDL [30]. LDL solutions were diluted to 0.30 g protein/liter with PBS containing 5 μmol/liter EDTA. Duplicate aliquots (100 μl) of the mixture were immediately taken, and 50 μl EDTA solution (3.7 mmol/liter) and 10 μl BHT solution (0.4 mmol/liter in methanol) were added. Next a volume (7 μl) of aqueous iodine solution (1 mmol/liter) and 86 μl acetic acid solution (3%) was added to the aliquots of LDL and to standards containing hydrogen peroxide and a blank of distilled water. One milliliter of a commercial iodide cholesterol color reagent (Merck Ltd) was added to all tubes and after 30 minutes in the dark at room temperature, the absorbance of the reaction mixture was read at 365 nm against the blank.

Vitamin E content of LDL expressed in μmol/g LDL protein was measured by high pressure liquid chromatography according to the method of Bieri, Tolliver and Catiguiani [31].

Statistical analysis

Comparison of values among groups of subjects were made using analysis of variances combined with Duncan's multiple range test. Student's *t*-test was used for comparing values between two groups of subjects. Pearson's product-moment correlation coefficients were used to test for relationships between variables. In one case, Spearman's rank correlation was used to reduce the effect of an outlying value on the correlation. Partial correlation analysis was used to test relationships between two variables while a third variable was held constant. Two-tailed tests of significance were used and a P value of less than 0.05 was considered to be statistically significant.

Results

Clinical characteristics and plasma lipids and lipoprotein concentrations of the subjects are shown in Table 1. The CAPD patients were significantly older than the healthy subjects and other renal patients. Plasma creatinine concentration was lower in renal transplant recipients compared with dialyzed patients with renal failure due to a successfully functioning kidney graft. Cholesterol concentration was significantly higher in plasma from

Table 2. Chemical composition and vitamin E content of low density lipoproteins in patients with renal disease

	HD $N = 13$	CAPD $N = 7$	RT $N = 18$	Healthy subjects $N = 15$	ANOVA P
Protein %	26.5 ± 4.0[bc]	24.0 ± 1.3	24.3 ± 1.3	24.8 ± 1.1	0.045
FC %	7.5 ± 0.9[ac]	8.5 ± 0.7[d]	10.0 ± 1.5	9.5 ± 2.2	< 0.001
CE %	40.1 ± 4.9	41.6 ± 2.4	41.3 ± 3.1	42.2 ± 2.6	NS
TG %	8.0 ± 2.2[ac]	7.9 ± 2.3[ad]	6.1 ± 1.4	5.1 ± 1.1	< 0.001
PL %	17.9 ± 2.0	18.1 ± 0.5	18.3 ± 2.1	18.4 ± 1.2	NS
Lipid peroxides *nmol/mg*	44 ± 9[abc]	25 ± 5	23 ± 7	24 ± 8	< 0.001
Vitamin E *nmol/mg protein*	5.3 ± 1.5	5.5 ± 2.6	3.9 ± 1.5[cd]	4.2 ± 1.4	0.05

Values are mean ± SD. Abbreviations are: HD, hemodialysis; CAPD, continuous ambulatory peritoneal dialysis; RT, renal transplant; FC, free cholesterol; CE, cholesteryl esters; TG, triglycerides; PL, phospholipids.

Significance $P < 0.05$ (Duncan's test): [a] vs. healthy subjects; [b] HD vs. CAPD; [c] HD vs. RT; [d] CAPD vs. RT

Table 3. Fatty acid composition of low density lipoproteins isolated from patients with renal disease

Fatty acid *wt %*	HD $N = 13$	CAPD $N = 7$	RT $N = 18$	Healthy subjects $N = 14$[a]	ANOVA P
16:0	18.3 ± 2.6	18.0 ± 1.9	16.1 ± 2.6	16.9 ± 2.6	NS
16:1	1.4 ± 0.5	1.9 ± 0.7	1.7 ± 0.7	1.5 ± 0.4	NS
18:0	10.6 ± 1.5	8.8 ± 1.2	9.0 ± 2.5	9.6 ± 1.7	NS
18:1	21.2 ± 3.6[b]	23.8 ± 0.5[bc]	20.6 ± 2.6[b]	18.2 ± 1.9	0.002
18:2 n-6	33.8 ± 5.7	31.1 ± 6.8[bc]	36.2 ± 4.8	37.4 ± 4.0	0.046
18:3 n-3	1.7 ± 0.5	1.9 ± 1.0	1.7 ± 0.6	1.9 ± 0.5	NS
20:3 n-6	1.4 ± 0.6	1.7 ± 0.5	1.6 ± 0.5	1.7 ± 0.4	NS
20:4 n-6	5.5 ± 1.9	6.5 ± 1.8	6.8 ± 1.6	6.4 ± 1.6	NS
20:5 n-3	2.3 ± 0.6	2.4 ± 1.2	2.8 ± 2.3	2.5 ± 1.5	NS
22:5 n-3	1.0 ± 0.9	0.7 ± 0.8	1.2 ± 1.0	1.6 ± 1.5	NS
22:6 n-3	2.7 ± 1.0	3.4 ± 1.0	2.6 ± 1.1	2.5 ± 0.9	NS

Values are mean ± SD. Abbreviations are: HD, hemodialysis; CAPD, continuous ambulatory peritoneal dialysis; RT, renal transplant.

[a] Data for one subject are missing for technical reasons

Significance $P < 0.05$ (Duncan's test): [b] vs. healthy subjects; [c] CAPD vs. RT

CAPD patients and renal transplant recipients compared with healthy controls and hemodialyzed patients whose values were similar to those in the controls. Plasma VLDL+IDL cholesterol levels were significantly higher in patients treated by dialysis compared with levels in the healthy subjects and renal transplant recipients. The concentration of plasma HDL cholesterol in hemodialyzed patients was significantly lower than levels in healthy subjects and renal transplant recipients. Patients treated by CAPD had levels of HDL cholesterol which were similar to those in hemodialyzed patients and were significantly lower than levels in renal transplant recipients. Plasma Lp(a) concentration tended to be higher but not significantly (ANOVA $P = 0.07$, using log transformed data) in patients receiving dialysis.

The chemical composition and content of lipid peroxides and vitamin E in LDL from the subjects are shown in Table 2. Patients treated by hemodialysis or CAPD had significantly lower content of free cholesterol and significantly higher content of triglycerides in LDL compared with renal transplant recipients and healthy subjects. The contents of protein and lipid peroxides were significantly higher in LDL from hemodialysis patients compared with other subjects. The vitamin E content in LDL from renal transplant recipients was significantly lower than levels in dialyzed patients with chronic renal failure. The ratio cholesterol/protein in LDL from hemodialysis patients (3.15 ± 0.73 μmol/mg) was significantly (ANOVA $P = 0.008$) lower compared with values from other subjects (CAPD 3.57 ± 0.36 μmol/mg; renal transplant recipients 3.68 ± 0.21 μmol/mg; healthy subjects 3.60 ± 0.26 μmol/mg).

The fatty acid composition of LDL from patients with renal disease and healthy subjects is shown in Table 3. Oleic acid (18:1) content of LDL was significantly higher in all treatment categories of patients with renal disease compared with healthy subjects. Linoleic acid (18:2) content of LDL was significantly lower in patients treated by CAPD and tended to be lower in hemodialyzed patients compared with healthy subjects.

Table 4 summarizes data obtained from the oxidation of isolated LDL by copper ions. The lag time in conjugated diene formation was significantly shorter in renal transplant recipients compared with healthy subjects and hemodialyzed patients. The rate of conjugated diene formation and the maximum quantity of diene production were significantly lower in all categories of renal patients compared with healthy subjects. When patients who were receiving cardiovascular drugs including ACE inhibitors, calcium channel blockers and β-blockers were excluded essentially similar findings were obtained. Also two-way analysis of variances by treatment group and cardiovascular drug therapy showed that there was no significant effect of cardiovascular drugs on LDL oxidation variables. Mean (± SD) levels of the LDL oxidation variables were not significantly different between renal transplant recipients treated with azathioprine and prednisone (lag time 75 ± 37 min; rate 8.29 ± 1.01 nmol/mg/min; concentration 620 ± 51 nmol/mg; $N = 5$) and those treated with cyclosporine and prednisone with or without azathioprine (lag time 62 ± 27 min; rate 8.27 ± 1.01 nmol/mg/min; concentration 586 ± 67 nmol/mg; $N = 13$). The mean (± SD) lag time was significantly ($P < 0.05$) lower in LDL from hemodialysis patients who smoked (78 ± 9 min, $N = 3$) compared with the corresponding value in those who did not smoke (95 ± 11 min, $N = 10$). The mean (± SD) lipid peroxide content of LDL (smokers 48 ± 13 nmol/mg; nonsmokers 42 ± 8 nmol/mg) was not significantly different between these groups of patients. Two of the renal transplant recipients, both men, smoked, but excluding their data did not significantly affect measures of LDL oxidation variables.

There was substantial individual variation in lag time in LDL oxidation in renal transplant recipients (Fig. 1), and those with shorter lag times outside the current range of values in healthy subjects were mainly women. Table 5 summarizes LDL oxidation and composition data in men and women treated by dialysis, with renal transplants and in the group of healthy subjects. Women with renal transplants had significantly shorter lag times in conjugated diene formation, lower LDL cholesteryl ester content and higher LDL triglyceride content compared with men with kidney grafts. Also the triglyceride content of LDL from these women was significantly ($P < 0.001$) higher than the corresponding value in healthy women. A significantly ($P = 0.047$) higher proportion of women with kidney grafts were treated with azathioprine and prednisone (22%), and a lower proportion were treated with cyclosporine in combination with prednisone and/or

Table 4. Conjugated diene formation during copper ion catalyzed oxidation of low density lipoproteins isolated from plasma of patients with renal disease

	HD	CAPD	RT	Healthy subjects	ANOVA *P*
All subjects	$N = 13$	$N = 7$	$N = 18$	$N = 15$	
Lag time *min*	91 ± 13	82 ± 15	66 ± 29[ac]	83 ± 17	0.011
Rate *nmol/min/mg*	7.44 ± 1.56[a]	7.43 ± 2.20[a]	8.28 ± 1.12[a]	9.44 ± 1.64	0.005
Concentration *nmol/mg*	571 ± 55[a]	562 ± 95[a]	596 ± 64[a]	671 ± 86	0.002
No cardiovascular drugs[d]	$N = 5$	$N = 7$	$N = 8$	$N = 15$	
Lag time *min*	86 ± 6	82 ± 15	58 ± 29[ac]	83 ± 17	0.023
Rate *nmol/min/mg*	7.46 ± 2.12	7.43 ± 2.20	8.20 ± 1.40	9.44 ± 1.64	0.052
Concentration *nmol/mg*	581 ± 66[a]	562 ± 95[a]	591 ± 80[a]	671 ± 86	0.024

Values are mean ± SD and apart from lag time, are expressed with respect to LDL protein.
Abbreviations are: HD, hemodialysis; CAPD, continuous ambulatory peritoneal dialysis; RT, renal transplant.
Significance $P < 0.05$ (Duncan's test): [a] vs. healthy subjects; [b] HD vs. CAPD; [c] HD vs. RT.
[d] Patients receiving angiotensin converting enzyme inhibitors, calcium channel blocking agents and β-blocking drugs were excluded.

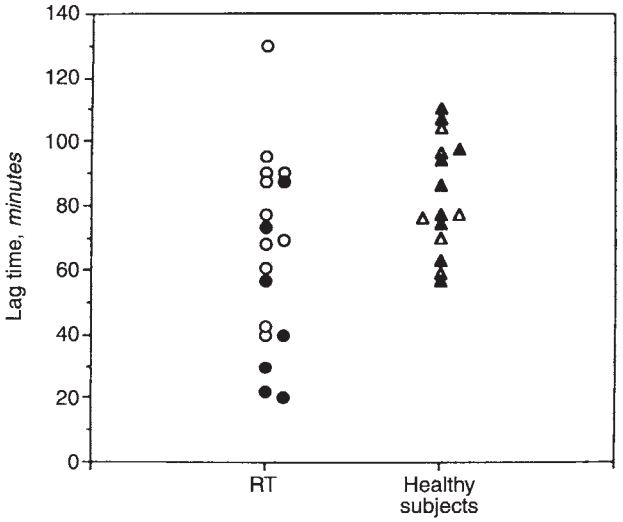

Fig. 1. *Lag time in conjugated diene formation during copper ion oxidation of low density lipoproteins isolated from renal transplant recipients (RT) and healthy subjects.* Men with renal transplants (○); women with renal transplants (●); healthy men (Δ); healthy women (▲).

azathioprine (17%) compared with men (6% and 56%, respectively). Mean whole blood cyclosporine levels were 166 ng/ml (range 62 to 256 ng/ml) in women and 212 ng/ml (range 125 to 398 ng/ml) in men. The corresponding mean plasma levels were 70 ng/ml (women) and 104 ng/ml (men). The lag time was lower in women receiving triple therapy (27 ± 11 min, $N = 3$) compared with those receiving double therapy (62 ± 24 min, $N = 4$) at a marginal level of significance ($P = 0.07$). The content of triglycerides in LDL was not significantly different between these two groups of women. In men with renal transplants, the lag time was not significantly different between those receiving triple therapy (73 ± 18 min, $N = 8$) compared with those treated with double therapy (88 ± 14 min, $N = 3$). The proportion of women with renal transplants taking cardiovascular drugs (43%) was not significantly different from the corresponding proportion of men (36%). Also, age (men 44 ± 14 years; women 40 ± 12 years; mean ± SD) and LDL content of oleic acid (men 20.5 ± 2.7%; women 20.6 ± 2.6%) were not significantly different between men and women with renal transplants. In women treated by dialysis (hemodialysis or CAPD), the lag time was significantly longer, the maximum concentration of dienes produced was significantly less, LDL triglyceride content was higher at a marginal level of significance ($P = 0.063$) and LDL vitamin E content was significantly higher compared with values in men receiving dialysis. The content of oleic acid in LDL tended to be higher (women 24.4 ± 3.2%; men 21.7 ± 4.1%; mean ± SD) and the content of linoleic acid tended to be lower (women 30.1 ± 7.1%; men 33.3 ± 6.0%; mean ± SD) in women receiving dialysis, but these differences did not attain statistical significance.

Table 6 shows the effect of hemodialysis on copper-catalyzed oxidation of isolated LDL from 12 out of the 13 patients treated by hemodialysis. None of the measured variables changed significantly during hemodialysis. Baseline lipid peroxide content of LDL also did not change significantly during hemodialysis (immediately before 45 ± 8 nmol/mg; end 49 ± 23 nmol/mg; 24 hr 46 ± 6 nmol/mg; mean ± SD).

Since the patients with evidence of IHD were all dialysis-dependent their characteristics were compared with those of the remaining dialysis-dependent patients who had no evidence of IHD, and these data are summarised in Table 7. The patients with IHD were significantly older than those with no evidence of the condition.

Correlation analysis

The lag time ($r = 0.357$, $N = 52$, $P = 0.009$), rate ($r = -0.670$, $N = 52$, $P < 0.001$) and maximum concentration ($r = -0.615$, $N = 52$, $P < 0.001$) of conjugated dienes formed were correlated significantly with the oleic acid content of LDL in all subjects combined (Fig. 2). The rate ($r = 0.627$, $N = 52$, $P < 0.001$) and maximum concentration ($r = 0.561$, $N = 52$, $P < 0.001$) of dienes formed were also correlated significantly with the linoleic acid content of LDL (Fig. 3). The lag time was correlated significantly with the triglyceride content of LDL in renal transplant recipients (Fig. 4) but not in the other groups of subjects (hemodialysis: $r = -0.164$; CAPD: $r = 0.276$; healthy subjects: $r = -0.190$). In those who were receiving "triple therapy," the lag time was not correlated significantly with the plasma concentration of cyclosporine ($r = 0.017$, $N = 13$). The vitamin E concentration in LDL ($r = 0.166$) and the rate of diene formation ($r = -0.117$) were not correlated significantly with the lag time in conjugated diene formation ($N = 53$). The rate of conjugated diene formation ($r = -0.377$, $N = 53$, $P = 0.005$) and the maximum concentration of conjugated dienes formed ($r = -0.403$, $N = 53$, $P = 0.003$) were correlated significantly with the LDL content of triglyceride. The

Table 5. Low density lipoprotein oxidation and composition in men and women with renal disease and in healthy subjects

	Dialysis patients		RT		Healthy subjects	
	Men $N = 17$	Women $N = 3$	Men $N = 11$	Women $N = 7$	Men $N = 6$	Women $N = 9$
Lag time *min*	85 ± 13	104 ± 10[a]	77 ± 25	47 ± 26[a]	80 ± 17	85 ± 19
Rate *nmol/min/mg*	7.67 ± 1.79	6.13 ± 0.66	8.44 ± 1.09	8.03 ± 1.22	9.76 ± 2.03	9.23 ± 1.41
Concentration *nmol/mg*	581 ± 64	492 ± 45[a]	599 ± 57	590 ± 78	699 ± 104	653 ± 73
Protein %	25.5 ± 3.7	26.1 ± 2.4	24.3 ± 1.3	24.3 ± 1.4	24.7 ± 1.1	24.9 ± 1.2
FC %	7.9 ± 1.0	7.8 ± 0.3	9.8 ± 1.7	10.4 ± 1.1	8.4 ± 1.5	10.3 ± 2.4
CE %	41.0 ± 4.3	38.3 ± 1.9	42.5 ± 3.0	39.5 ± 2.3[a]	43.7 ± 0.8	41.2 ± 2.9[a]
TG %	7.6 ± 1.9	10.1 ± 2.8	5.3 ± 0.8	7.3 ± 1.2[b]	5.2 ± 1.4	5.0 ± 0.9
PL %	18.0 ± 1.8	17.8 ± 0.1	18.5 ± 2.7	18.4 ± 2.5	18.0 ± 1.3	18.7 ± 1.1
Lipid peroxides *nmol/mg*	36 ± 13	40 ± 12	23 ± 5	23 ± 9	24 ± 3	26 ± 6
Vitamin E *nmol/mg*	5.0 ± 1.5	7.4 ± 3.0[a]	3.5 ± 1.4	4.4 ± 1.7	4.1 ± 1.4	4.2 ± 1.4

Values are mean ± SD. Abbreviations are: RT, renal transplant recipients; FC, free cholesterol; CE, cholesteryl esters; TG, triglycerides; PL, phospholipids.

Significance of difference between mean values for men and women (Student's *t*-test): [a] $P < 0.05$; [b] $P < 0.001$

Table 6. Effect of hemodialysis therapy on conjugated diene formation during copper ion catalyzed oxidation of low density lipoproteins from patients with renal disease

	States of hemodialysis			ANOVA
	Before[a]	End	24 hr later	*P*
Lag time *min*	90 ± 12	88 ± 32	86 ± 29	NS
Rate *nmol/mg/h*	7.21 ± 1.39	6.86 ± 1.81	6.75 ± 1.35	NS
Maximum *nmol/mg*	563 ± 49	541 ± 50	566 ± 52	NS

Values are mean ± SD, $N = 12$.

[a] "Before" is immediately prior to dialysis and "end" is immediately after dialysis

Table 7. Characteristics of patients with renal disease and ischemic heart disease (IHD[+]) and patients undergoing similar therapy but with no evidence of ischemic heart disease (IHD[−])

	IHD[+][a] $N = 8$	IHD[−] $N = 12$	*P*
Clinical features			
Age *years*	67 ± 7	49 ± 18	0.005
Body weight *kg*	68.7 ± 8.8	68.5 ± 11.7	NS
Duration of treatment *months*	17 ± 12	43 ± 40	0.052
Plasma creatinine *μmol/liter*	688 ± 169	782 ± 293	NS
LDL oxidation			
Lag time *min*	91 ± 12	86 ± 15	NS
Rate *nmol/min/mg*	7.36 ± 1.89	7.49 ± 1.74	NS
Concentration *nmol/mg*	586 ± 74	556 ± 66	NS
Plasma lipids and lipoproteins *mmol/liter*			
Cholesterol	611 ± 1.23	5.79 ± 1.62	NS
VLDL + IDL cholesterol	1.30 ± 0.64	1.59 ± 0.99	NS
LDL cholesterol	3.45 ± 0.99	2.86 ± 0.89	NS
HDL cholesterol	1.12 ± 0.40	0.95 ± 0.47	NS
Triglycerides	1.76 ± 0.86	2.29 ± 0.90	NS
VLDL + IDL triglycerides	1.24 ± 0.77	1.80 ± 0.77	NS
Lp(a) *U/liter*	574 ± 530	479 ± 413	NS[b]

Values are mean ± SD. Abbreviations are: VLDL, very low density lipoproteins; IDL, intermediate density lipoproteins; LDL, low density lipoproteins; HDL, high density lipoproteins.

[a] Includes 4 patients treated by hemodialysis and 4 patients treated by CAPD.

[b] Significance calculated on log transformed data

content of triglycerides in LDL was also correlated significantly with the corresponding content of oleic acid ($r = 0.398$, $N = 52$, $P = 0.004$) and linoleic acid ($r = -0.415$, $N = 52$, $P = 0.002$). When LDL content of oleic acid or linoleic acid was held constant in partial correlation analysis, the rate of diene formation (oleic acid $r = -0.162$; linoleic acid $r = -0.165$) and maximum concentration of dienes produced (oleic acid $r = -0.218$; linoleic acid $r = -0.226$) were no longer correlated significantly with LDL triglycerides. The lag time was not correlated significantly with duration of treatment in patients receiving hemodialysis ($r = -0.221$) or CAPD ($r = -0.130$) and renal transplant recipients ($r = 0.000$).

Discussion

Oxidation of LDL is believed to be an important step in the development of atherosclerosis [8]. Since atherosclerotic disease is prevalent in patients with chronic renal failure or renal transplants, we sought evidence that LDL from these patients is more susceptible to oxidation *in vitro*. Our data show that lag times in conjugated diene formation are abnormally short when LDL from women with renal transplants are oxidized *in vitro*, suggesting that the susceptibility of the lipoprotein to oxidation may be increased in these women.

The inverse correlation between lag time and LDL triglyceride content in the present renal transplant recipients suggests that the short lag time in LDL oxidation in women with kidney grafts may be due in part to the abnormally high content of triglycerides in the lipoprotein. Triglyceride-enrichment of LDL has also been associated with increased susceptibility to copper ion oxidation in male survivors of myocardial infarction in a previous study [10]. The mechanism underlying this association is not clear. It is possible that triglyceride-enrichment may alter the physicochemical properties of LDL and thereby increase the susceptibility of the lipoprotein to oxidation. In the present study, LDL from men and women receiving dialysis treatment were also triglyceride-enriched but lag times in LDL oxidation were not reduced. This finding may be due to increased antioxidant protection as a result of the higher content of vitamin E in LDL from these patients compared with the renal transplant recipients. Vitamin E is the most abundant antioxidant in LDL and raising the levels of this antioxidant increases the lag time in LDL oxidation [32].

Cyclosporine at blood levels greater than 100 ng/ml is reported as accelerating the oxidation of isolated LDL [20]. In the present

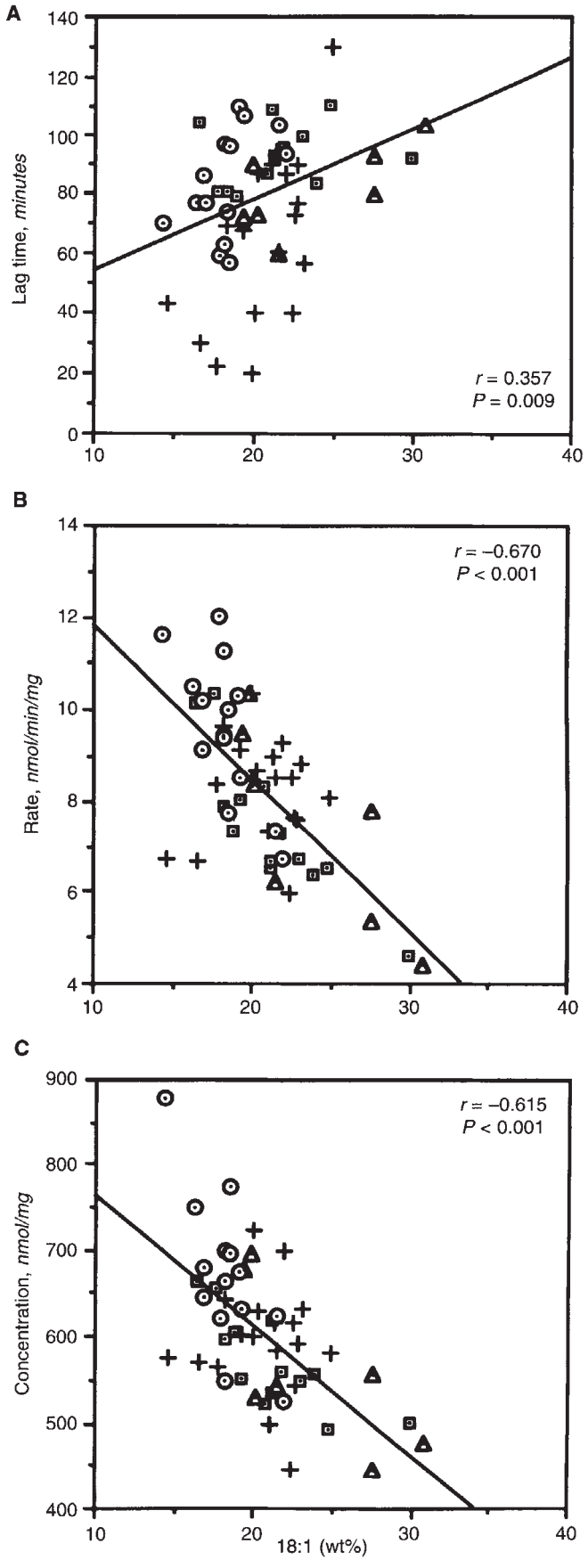

Fig. 2. *Correlation between low density lipoprotein (LDL) oleic acid (18:1) content and lag time, rate and maximum concentration in conjugated diene formation during copper ion oxidation of LDL isolated from patients with chronic renal failure or a renal transplant.* Symbols are: (□) hemodialysis (N = 13); (Δ) CAPD (N = 7); (+) renal transplant recipients (N = 18); (○) healthy subjects (N = 14).

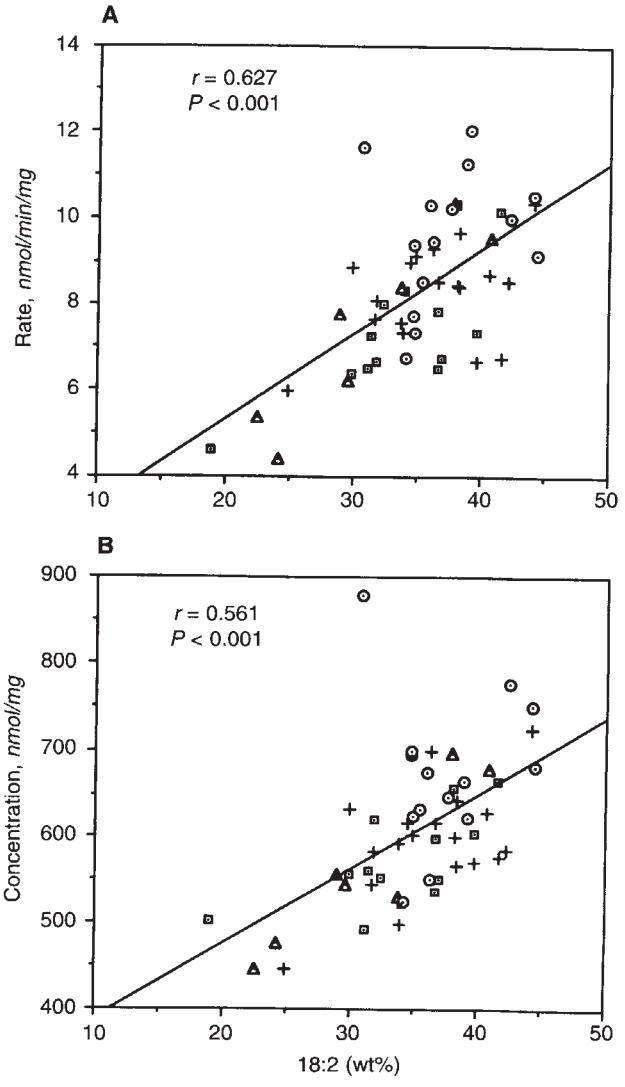

Fig. 3. *Correlation between low density lipoprotein linoleic acid content (18:2) and rate and maximum concentration of conjugated dienes formed during copper ion oxidation of LDL isolated from patients with chronic renal failure or a renal transplant.* Symbols are: (□) hemodialysis (N = 13); (Δ) CAPD (N = 7); (+) renal transplant recipients (N = 18); (○) healthy subjects (N = 14).

study, LDL from women receiving triple therapy tended to be particularly susceptible to oxidation, suggesting that cyclosporine treatment may have contributed to the short lag time in LDL oxidation in women with kidney grafts. However, plasma cyclosporine levels were unrelated to LDL oxidation in renal transplant recipients and cyclosporine treatment did not appear to have greatly influenced the oxidation of LDL from men with kidney grafts. They had lag times in LDL oxidation that were not clearly

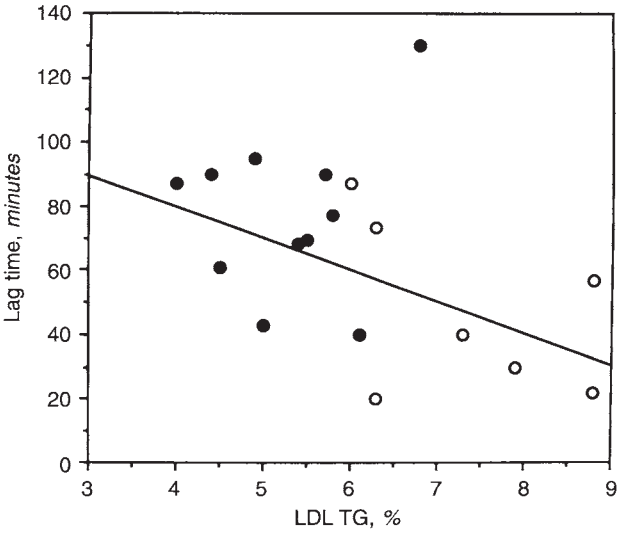

Fig. 4. *Correlation between low density lipoprotein triglyceride content (LDL TG) and lag time in conjugated diene formation during copper ion oxidation of LDL isolated from renal transplant recipients.* Symbols are: (●) men ($N = 11$); (○) women ($N = 7$). Spearman's rank correlation coefficient for the relationship is -0.502, $P = 0.034$.

different from normal in spite of the fact that their blood cyclosporine levels were higher than levels (100 ng/ml) reported as stimulating LDL oxidation *in vitro* [20]. This apparent ineffectiveness of cyclosporine treatment on LDL oxidation in men is unexplained. Although captopril (an ACE inhibitor) and β-blocking drugs are reported as reducing the susceptibility of isolated LDL to oxidation [33, 34], there was no evidence in our data that treatment with cardiovascular drugs had any appreciable influence on LDL oxidation in renal transplant recipients or in the total group of renal patients.

In this study and in a previous report [21], the lag time in conjugated diene formation was linked with LDL oleate content, but this relationship did not appear to explain the abnormally short lag times in oxidation of LDL from the present women with renal transplants. Thus the fatty acid composition of LDL was similar in men and women with kidney grafts while lag times were markedly longer in the men. Once antioxidant protection was depleted, the rate and extent of conjugated diene formation from LDL isolated from renal patients, and more particularly those treated by dialysis, were lower than values in healthy subjects. The close correlations between these oxidation variables and LDL content of oleic acid (inverse) and linoleic acid (positive) suggest that lower LDL linoleate and higher LDL oleate content contributed to the lower rate and extent of conjugated diene formation in LDL from dialysis patients. Similar relationships have been reported previously in mildly hypercholesterolemic patients [22] and a combined group of healthy subjects and vitamin E-deficient individuals [21]. Furthermore, changes in LDL content of oleate and linoleate by supplementing the diet with these fatty acids produces the predicted changes in rate and extent of LDL oxidation [22–24]. Usually a faster maximum rate accompanies a shorter lag time in LDL oxidation, but in the present renal transplant recipients the lag time was shorter and the rate of diene formation was slower than normal. This apparent discrepancy may be due to comparatively high LDL oleate content which is associated with slower rates of diene formation.

In contrast to a recent report of increased susceptibility to oxidation of LDL isolated from dialysis patients [19], LDL from the corresponding patients in the present study was not abnormally prone to oxidation. Whether or not these differing findings are related to the shorter lag times in conjugated diene formation in LDL from the dialysis patients and healthy subjects we studied compared with those reported previously [19] is uncertain. Vitamin E content of LDL was lower and may explain the shorter lag times in our data. Also, the patients reported on by Maggi et al [19] were from Italy where the diet is usually rich in olive oil, and could therefore lead to LDL that is enriched in oleic acid and particularly resistant to oxidation [22–24]. It seems unlikely that oxidation of LDL during its separation from plasma is mainly responsible for the shorter lag times we observed because EDTA was present throughout the isolation procedure. Lipid peroxide levels were abnormally high in LDL from the current hemodialysis patients, but this finding has been documented previously [17]. There is evidence that enrichment with lipid peroxides increases the susceptibility of LDL to oxidation by copper ions [35, 36]. However, in spite of increased lipid peroxide content hemodialyzed LDL was not abnormally susceptible to oxidation in the present study. It is possible that the higher vitamin E content and lower cholesterol/protein ratio may have counteracted any prooxidant effects of raised lipid peroxide levels in hemodialyzed LDL. In support of this hypothesis, Frei and Gaziano [36] have reported that a low cholesterol/protein ratio is associated with increased resistance of LDL to oxidation by copper ions. In the present study, the comparatively normal resistance of hemodialyzed LDL to oxidation seems to be consistent with the finding that acute hemodialysis did not alter the susceptibility of LDL to oxidation, and the duration of hemodialysis treatment and the lag time in LDL oxidation were unrelated. These variables were also unrelated in the study reported by Maggi et al [19].

Acute smoking is reported as raising LDL lipid peroxide content and increasing the susceptibility of the isolated lipoprotein to oxidation [37]. Accordingly, LDL from current hemodialysis patients who smoked showed increased susceptibility to oxidation, but this did not appear to be mediated by lipid peroxide content of the lipoprotein which was similar in smokers and nonsmokers. Overall, there were few smokers and smoking status did not appreciably influence major findings on LDL oxidation in this study.

We isolated plasma LDL between densities 1.019 g/ml and 1.063 g/ml and the lipoprotein fraction may therefore contain varying quantities of Lp(a) that has a longer lag time than LDL during copper ion oxidation [27]. It is unlikely that Lp(a) content of the LDL fraction greatly influenced the lag times in diene formation that we observed in renal patients, because lag times were similar in CAPD patients and healthy controls despite higher levels of plasma Lp(a) in the patients.

In the present study, the maximum rates of LDL oxidation suggest that LDL from all patients with renal disease, and particularly those receiving dialysis, may be less readily oxidized than normal. In contrast, lag times in LDL oxidation suggest that the susceptibility of the lipoprotein to oxidation is comparatively normal in most patients with renal disease, excepting women with renal transplants whose LDL is more prone to oxidation. This inconsistency raises the question which index of LDL oxidation

should be given greater weight in assessing the potential of LDL for oxidation *in vivo*. De Graaf and coworkers [38] have suggested that more value should be given to the duration of the lag time in conjugated diene formation because at the end of this period there are alterations in LDL that could theoretically influence its biological activity. Indeed, increased levels of minimally oxidatively modified LDL would be expected during or not long after the end of the lag time, and these particles have potentially atherogenic properties [39]. On the other hand, if an LDL particle becomes trapped within the intimal matrix and subjected to sustained oxidative stress, the rate and extent of LDL oxidation may also be important in determining the subsequent likelihood and degree of any atherosclerotic changes. Recent evidence suggests that oxidation of LDL *in vitro* may not necessarily parallel *in vivo* oxidation of the lipoprotein measured as circulating levels of autoantibodies to oxidized LDL [40]. Increased levels of these antibodies have been detected in patients receiving dialysis [41], which seems to be at variance with our finding of normal susceptibility to *in vitro* oxidation of LDL isolated from this category of patients. Further studies comparing these measures of LDL oxidation are required.

Regnström and coworkers have reported that the lag time in LDL oxidation is correlated inversely with the severity of coronary atherosclerosis in men with a first myocardial infarction [10]. However, the presence of subjects with evidence of IHD did not appear to influence our findings, and values for LDL oxidation variables were similar in dialysis dependent patients with chronic renal failure with or without evidence of IHD, and none of the women with renal transplants had clinically important cardiovascular disease. A recent study has reported similar levels of circulating autoantibodies to oxidized LDL, suggesting that rates of LDL oxidation *in vivo* may also be similar in dialysis patients with or without IHD [41]. It is possible that subclinical vascular disease was present in patients without evidence of IHD, and that there were differences in lifestyle that may have obscured any difference in LDL oxidation associated with IHD. On the other hand, it is also possible that LDL oxidation is not the predominant risk factor for IHD in dialysis patients. The classical risk factors including older age and higher plasma LDL cholesterol levels tended to distinguish patients with evidence of IHD in our data.

There are limitations to the present findings. The main study was cross sectional, and levels of variables prior to treatment for kidney failure are unknown. The numbers of subjects in each category of renal patients were comparatively small, increasing the risk of a non-representative sample. On the other hand, clinical findings and plasma lipid and lipoprotein levels in patients in the treatment groups were essentially in agreement with reported data [4–6]. The trend toward high levels of HDL cholesterol in the renal transplant recipients we studied has been noted in some [42], but not all studies [43, 44], and is possibly due to immunosuppressive therapy and improved renal function [42].

In conclusion, this study has provided evidence that LDL from women with renal transplants is abnormally susceptible to oxidation *in vitro*, and we speculate that this may possibly increase their risk of future atherosclerotic disease. Measurement of autoantibodies against oxidized LDL is required to confirm that oxidation of LDL *in vivo* may also be increased in these women.

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References

1. US RENAL DATA SYSTEM USRDS 1991: *Annual Data Report*. Bethesda, The National Institute of Health, National Institute of Diabetes and Digestive and Kidney Diseases, 1991
2. LINDER A, CHARRA B, SHERRARD DJ, SCRIBNER BH: Accelerated atherosclerosis in prolonged maintenance hemodialysis. *N Engl J Med* 290:698–701, 1974
3. KASISKE BL: Risk factors for accelerated atherosclerosis in renal transplant recipients. *Am J Med* 84:985–992, 1988
4. ATTMAN P-O, ALAUPOVIC P: Lipid abnormalities in chronic renal insufficiency. *Kidney Int* 39 (Suppl 31):S16–S23, 1991
5. SENTI M, ROMERO R, PEDRO-BOTET J, PELEGRI A, NOGUE X, RUBIES-PRAT J: Lipoprotein abnormalities in hyperlipidemic and normolipidemic men on hemodialysis with chronic renal failure. *Kidney Int* 41:1394–1399, 1992
6. DRUEKE TB, ABDULMASSIH Z, LACOUR B, BADER C, KREIS H: Atherosclerosis and lipid disorders after renal transplantation. *Kidney Int* 39 (Suppl 31):S24–S28, 1991
7. STRINGER MD, GÖRÖG PG, FREEMAN A, KAKKAR VV: Lipid peroxides and atherosclerosis. *Brit Med J* 298:281–284, 1989
8. STEINBERG D, PARTHASARATHY S, CAREW TE, KHOO JC, WITZTUM JL: Beyond cholesterol. Modifications of low density lipoprotein that increases its atherogenicity. *N Engl J Med* 320:915–923, 1989
9. SALONEN JT, YLÄ-HERTTUALA S, YAMAMOTO R, BUTLER S, KORPELA H, SALONEN R, NYYSSONEN K, PALINSKI W, WITZTUM JL: Autoantibodies against oxidised LDL and progression of carotid atherosclerosis. *Lancet* 339:883–887, 1992
10. REGNSTRÖM J, NILSSON J, TORNVALL P, LANDON C, HAMSTEN A: Susceptibility of low density lipoprotein to oxidation and atherosclerosis. *Lancet* 339:1183–1186, 1992
11. CAREW TE, SCHWENKE DC, STEINBERG D: Antiatherogenic effect of probucol unrelated to its hypocholesterolemic effect: Evidence that antioxidants in vivo can selectively inhibit low density lipoprotein degradation in macrophage-rich fatty streaks slowing the progression of atherosclerosis in the WHHL rabbit. *Proc Natl Acad Sci USA* 84:7725–7729, 1987
12. BJORKHAM I, HENRIKSSON-FREYSCHUSS A, BREUER O, DICZFALUSY U, BERGLUND L, HENRIKSSON P: The antioxidant butylated hydroxytoluene protects against atherosclerosis. *Arterioscl Thromb* 11:15–22, 1991
13. MOREL DW, DI CORLETO PE, CHISOLM GM: Endothelial and smooth muscle cells alter low density lipoprotein in vitro by free radical oxidation. *Arteriosclerosis* 4:357–364, 1984
14. QUINN MT, PARTHASARATHY S, FONG L, STEINBERG D: Oxidatively modified low density lipoproteins: A potential role in recruitment and retention of monocyte/macrophages during atherogenesis. *Proc Natl Acad Sci USA* 84:2995–2998, 1987
15. FILLIT H, ELION E, SULLIVAN J, SHERMAN R, ZABRISKIE JB: Thiobarbituric acid reactive material in uremic blood. *Nephron* 29:40–43, 1981
16. RICHARD MJ, ARNAUD J, JURKOVITZ C, HACHEACHE T, MEFTAHI H, LAPOTE F, FORET M, FAVIER A, CORDONNIER D: Trace elements and lipid peroxidation abnormalities in patients with chronic renal failure. *Nephron* 57:10–15, 1991
17. TOBOREK M, WASIK T, DRÓZDZ M, KLIN M, MAGNER-WRÓBEL K, KOPIECZNA-GRZEBIENIAK E: Effect of hemodialysis on lipid peroxidation and antioxidant system in patients with chronic renal failure. *Metabolism* 41:1229–1232, 1992
18. NGUYEN AT, LETHIAS C, ZINGRAFF J, HERBELIN H, NARET C, DESCAMPS-LATSCHA B: Hemodialysis membrane-induced activation of phagocyte oxidative metabolism detected in vivo and in vitro within micro amounts of whole blood. *Kidney Int* 28:158–167, 1985

19. MAGGI E, BELLAZZI R, FALASCHI F, FRATTONI A, PERRANI G, FINARDI G, GAZO A, NAI M, ROMANINI D, BELLOMO G: Enhanced LDL oxidation in uremic patients: An additional mechanism for accelerated atherosclerosis? *Kidney Int* 45:876–883, 1994
20. APANAY DC, NEYLAN JF, RAGAB MS, SGOUTAS DS: Cyclosporine increases the oxidizability of low-density lipoproteins in renal transplant recipients. *Transplantation* 58:663–669, 1994
21. KLEINVELD HA, NABER AHJ, STALENHOEF AFH, DEMACHER PNM: Oxidation resistance, oxidation rate and extent of oxidation of human low-density lipoprotein depend on the ratio of oleic acid content to linoleic acid content: Studies in vitamin E deficient subjects. *Free Radical Biol Med* 15:273–280, 1993
22. REAVEN P, PARTHASARATHY S, GRASSE BJ, MILLER E, STEINBERG D, WITZTUM JL: Effects of oleate-rich and linoleate-rich diets on the susceptibility of low density lipoproteins to oxidative modification in mildly hypercholesterolemic subjects. *J Clin Invest* 91:668–676, 1993
23. ABBEY M, BELLING GB, NOAKES M, HIRATA F, NESTEL PJ: Oxidation of low-density lipoproteins: intraindividual variability and the effect of dietary linoleate supplementation. *Am J Clin Nutr* 57:391–398, 1993
24. BONANOME A, PAGNAN A, BIFFANTI S, OPPORTUNO A, SORGATO F, DORELLA M, MAIORINO M, URSINI F: Effect of dietary monounsaturated and polyunsaturated fatty acids on the susceptibility of plasma low density lipoproteins to oxidative modification. *Arterioscler Thromb* 12:529–533, 1992
25. LOWRY OH, ROSEBROUGH NJ, FARR AL, RANDALL RJ: Protein measurement with the Folin phenol reagent. *J Biol Chem* 193:265–275, 1951
26. WARNICK GR, BENDERSON J, ALBERS JJ: Dextran-sulphate-Mg^{2+} precipitation procedure for quantification of high density lipoprotein cholesterol. *Clin Chem* 28:1379–1388, 1982
27. SATTLER W, KOSTNER GM, WAEG G, ESTERBAUER H: Oxidation of Lp(a): A comparison with low density lipoproteins. *Biochim Biophys Acta* 1081:65–74, 1991
28. FOLCH J, LEES M, SLOANE-STANLEY GH: A simple method for the isolation and purification of total lipids from animal tissues. *J Biol Chem* 226:497–509, 1957
29. EL-SAADANI M, ESTERBAUER H, EL-SAYED M, GOHER M, NASSAR AY, JURGENS G: A spectrophotometric assay for lipid peroxides in serum lipoproteins using a commercially available reagent. *J Lipid Res* 30:627–630, 1989
30. BABIY AV, GEBICKI JM, SULLIVAN DR: Vitamin E content and low density lipoprotein oxidisability induced by free radicals. *Atherosclerosis* 81:175–182, 1990
31. BIERI JG, TOLLIVER IJ, CATIGUIANI GL: Simultaneous determination of alpha-tocopherol and retinol in plasma or red cells by high pressure liquid chromatography. *Am J Clin Nutr* 32:2143–2149, 1979
32. DIEBER-ROTHENEDER M, PUHL H, WAEG G, STRIEGL G, ESTERBAUER H: Effect of oral supplementation with D-α-tocopherol on the vitamin E content of human low density lipoproteins and resistance to oxidation. *J Lipid Res* 32:1325–1332, 1991
33. GODFREY EG, STEWART J, DARGIE HJ, REID JL, DOMINICZAK M, HAMILTON CA, MCMURRAY J: Effects of ACE inhibitors on oxidation of human low density lipoprotein. *Br J Clin Pharmac* 37:63–66, 1994
34. CROFT KD, DIMMITT SB, MOULTON C, BEILIN LJ: Low density lipoprotein composition and oxidisability in coronary disease-apparent favourable effect of beta blockers. *Atherosclerosis* 97:123–130, 1992
35. O'LEARY VJ, DARLEY-USMAR VM, RUSSELL IJ, STONE D: Prooxidant effects of lipoxygenase-derived peroxides on the copper-initiated oxidation of low-density lipoprotein. *Biochem J* 282:631–634, 1992
36. FREI B, GAZIANO JM: Content of antioxidants, preformed lipid hydroperoxides, and cholesterol as predictors of the susceptibility of human LDL to metal ion-dependent and -independent oxidation. *J Lipid Res* 34:2135–2145, 1993
37. HARATS D, BEN-NAIM M, DABACH Y, HOLLANDER G, STEIN O, STEIN Y: Effect of vitamin C and E supplementation on susceptibility of plasma lipoproteins to peroxidation induced by acute smoking. *Atherosclerosis* 85:47–54, 1990
38. DE GRAAF J, HENDRIKS JCM, DENMACKER PNM, STALENHOEFF AFH: Identification of multiple dense LDL subfractions with enhanced susceptibility to in vitro oxidation among hypertriglyceridemic subjects. Normalization after clofibrate treatment. *Arterioscl Thromb* 13:712–719, 1993
39. BERLINER JA, TERRITO M, SEVANIAN A, RAMIN S, KIM J, BAMSHAD B, ESTERSON M, FOGELMAN AM: Minimally modified LDL stimulates monocyte endothelial interaction. *J Clin Invest* 85:1260–1266, 1990
40. MAGGI E, CHIESA R, MELISSANO G, CASTELLANO R, ASTORE D, GROSSI A, FINARDI G, BELLOMO G: LDL oxidation in patients with severe carotid atherosclerosis. A study of in vitro and in vivo oxidation markers. *Arterioscler Thromb* 14:1892–1899, 1994
41. MAGGI E, BELLAZZI R, GAZO A, SECCIA M, BELLOMO G: Autoantibodies against oxidatively-modified LDL in uremic patients undergoing dialysis. *Kidney Int* 46:869–876, 1994
42. ABBOTT LK, ELLIOT C, AITKEN JM, SOMER JB: Serum lipoprotein abnormalities in renal allograft recipients. *Atherosclerosis* 30:97–107, 1978
43. JUNG K, NEUMANN R, SCHOLZ D, NUGEL E: Abnormalities in the composition of serum high density lipoprotein in renal transplant recipients. *Clin Nephrol* 17:191–194, 1982
44. BAGDADE JD, ALBERS JJ: Plasma high-density lipoprotein concentrations in chronic-hemodialysis and renal-transplant patients. *N Engl J Med* 296:1436–1439, 1977